Hair Cell Regeneration: Where Are We and How Did We Get Here?

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Sensorineural Hearing Loss

A majority of the hearing loss in humans is due to the loss of or damage to the hair cells in the organ of Corti.

These cells are made only during embryonic development, so any loss or damage in a mature ear cannot be repaired.
In 1967, Bob Ruben showed that hair cells are produced only during a limited time, embryonic days 13-15, during mouse cochlear development. After this, no new cells are generated. Thus, any loss of hair cells in a mature mammalian ear leads to permanent hearing loss.

Ruben, RJ, Acta Otol Suppl 220, 1967
In 1988, studies from my lab in collaboration with Jeff Corwin and from Brenda Ryals and Ed Rubel demonstrated that mature birds could regenerate their cochlear hair cells following noise damage or aminoglycoside treatment.
Hair Cell Regeneration in the Bird Cochlea

It was proposed that an understanding of how this structural and functional regeneration occurs in the avian cochlea might lead to the development of therapies for treatment of sensorineural hearing loss in humans.
Regeneration After Sound Damage

Cotanche, Hear Res 30, 181-197, 1987
Where Are the New HCs Coming From?

Studies of DNA synthesis in these regenerating ears showed that the new hair cells were arising from formerly quiescent supporting cells that had re-entered the cell cycle, divided and produced new cells that then differentiated into hair cells and supporting cells to repair the damaged sensory epithelium.
Mitosis is Not The Sole Source of New Hair Cells

In 1996 reports from David Roberson and Henry Adler demonstrated that some new hair cells arose not from mitosis, but from supporting cells that changed their gene expression and began to differentiate directly as hair cells. This was termed Direct Transdifferentiation.

Adler & Raphael, Neurosci Lettr 205:17-20, 1996
Roberson et al., Aud Neurosci 2:195-205, 1996
Regeneration in the Avian Cochlea - Continuing Studies

We have continued to explore regeneration in the bird cochlea to determine how the process is initiated, regulated, and terminated.

Issues:

1. How does hair cell death control regeneration?

2. Why are two mechanisms (DT and Mitosis) needed and how do they differ?

3. What other animals are capable of regenerating hair cells?
Hair Cells Die by Apoptosis

Studies in bird vestibular and cochlear organs have demonstrated that gentamicin treatment induces hair cell death by apoptosis.

(Matsui et al., 2002, 2003; Cheng et al, 2003; Mangiardi et al., 2004; Duncan et al., 2006)
The Apoptotic Cascade in Hair Cells

1. **Initiation Stage** - **TIAR**
   - 12h after gent
   - Before any signs of damage to the hair cells

2. **Activation Stage** - **Cytochrome c**
   - 30-36h after gent
   - As hair cells begin to show damage

3. **Execution Stage** - **Caspase-3**
   - 36-48h after gent
   - As hair cells begin to be ejected

Mangiardi et al., J Comp Neurol 475:1-18, 2004
Time Course of Hair Cell Apoptosis in the BP
Hair Cell Death Regulates Supporting Cell Regeneration

Steps in the hair cell death pathway regulate the entrance and progression of adjacent supporting cells into regeneration

1. Signals from hair cells at the onset of cell death cause supporting cells to begin the regeneration process but then hold them at this point until the hair cells are ejected

2. Signals at the time of hair cell ejection allow the supporting cells to complete the regeneration process
Supporting Cell Tools for Regeneration

The supporting cells that are stimulated by hair cell death to undergo regeneration use two distinct mechanisms for producing new hair cells:

- **Direct Transdifferentiation**
- **Mitotic Proliferation**
Direct Transdifferentiation

A supporting cell changes its gene expression and becomes a hair cell without dividing.

This results in the gain of one hair cell at the expense of losing one supporting cell. In general there are 3-4 supporting cells for every hair cell in the sensory epithelium, so this affords a limited supply of replacement cells.
Mitotic Regeneration

After a dying hair cell is ejected, an adjacent supporting cell leaves quiescence, and re-enters the cell cycle.

The dividing cell gives rise to two or more daughter cells which then go on to differentiate into new hair cells and new supporting cells. This method replaces the lost hair cells but also replaces the supporting cells that left quiescence to undergo Mitosis or Direct Transdifferentiation.
Math1 in Direct Transdifferentiation

Supporting cells in the cochlea begin to express Math1 as early as 9h after gentamicin, which is 3h after the gentamicin reaches the hair cells and 3h before we see the first signs of hair cells beginning apoptosis.
Time Course of Direct Transdifferentiation
Time Course of S phase during Mitotic Regeneration

Duncan et al., J Comp Neurol 499:691-701, 2006
DT Precedes Mitosis

Roberson et al., J Neurosci Res 78:461-471, 2004
A Two Step Regeneration Mechanism

• The first new hair cells to arise (days 4-6) are from Direct Transdifferentiation.

• When more than a few hair cells are needed, the supporting cells begin to proliferate (days 3-5) to make more new cells.

• Some of these new cells become hair cells (days 6-10), while others become supporting cells to replace those lost to mitosis and Direct Transdifferentiation.
Both Direct Transdifferentiation and Mitotic Regeneration utilize the same developmental pathway to make a new hair cell once a common progenitor cell has been generated.

+1 HC, -1 SC

+1 HC, +1 SC
Summary - Avian Hair Cell Regeneration

1. Does not occur in the normal, undamaged ear
2. Is initiated by the onset of hair cell death pathways
3. Uses two distinct methods, one for rapid response, one for more extensive repair
4. Both use the same program for making new hair cells
5. Knows when to stop, i.e., produces only enough new hair cells and supporting cells to replace those that were lost
6. Is correlated with regeneration in the TM, Tegmentum Vasculosum and cochlear nerve terminals.
7. Leads to appropriate functional recovery
Regeneration in Other Vertebrates

Hair cell regeneration is not unique to birds. All other vertebrates exhibit regeneration in their inner ears and/or lateral line organs.

The mammalian cochlea is the only inner ear sensory epithelium that shows no regeneration in the mature organ of Corti.
Regeneration in Fish and Amphibians

Fish and amphibians exhibit constant growth in their sensory epithelia and an increase in hair cell numbers throughout life (Corwin, 1981, 1983, 1985; Popper & Hoxter, 1984)

Amphibian lateral line organs regenerate after damage by seeding of new neuromasts with precursor cells from surviving ones (Stone, 1933, 1937; Jones & Corwin, 1993, 1996)
Regeneration in Avians

Avian vestibular organs constantly turn over their hair cells so that old hair cells die at the same rate new ones are born. Thus, the size of the endorgan and number of hair cells remains constant throughout adult life.

The rate of regeneration in vestibular organs can be up-regulated following trauma or aminoglycoside damage.

Hair cell turnover does not normally exist in the mature avian cochlea; it is only initiated following the loss of mature hair cells.
Regeneration in Mammals

There is evidence of a small but significant amount of non-mitotic hair cell regeneration in mammalian vestibular organs.

The embryonic mammalian cochlea has the capability of non-mitotic regeneration during early development of the organ of Corti, but this is lost later in development.

The mature organ of Corti exhibits no evidence of being able to regenerate hair cells on its own after trauma.
Why not?
Why Can’t the Mammalian Cochlea Regenerate?

Both mammalian and avian cochleae undergo common developmental steps for differentiating hair cells from supporting cells.

In the chick, supporting cells recapitulate the developmental sequence to regenerate hair cells.

Supporting cells in the mammal cochlea are not normally able to re-enter the cell cycle when hair cells are lost or damaged. Nor can they undergo direct transdifferentiation.
Can We Make the Mammalian Cochlea Regenerate?

The presentations of my fellow panel members Ed Rubel, Stefan Heller, and Andy Groves will address this question by describing the various ways they have explored the potential for inducing regeneration in the mammalian inner ear.
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